Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 525–529

Establishment of Reference Ranges for Cortisol in Neonates, Infants, Children and Adolescents

By *L. Jonetz-Mentzel* and *Gudrun Wiedemann*

Klinisch-Chemisches Labor der Klinik und Poliklinik für Kindermedizin der Medizinischen Hochschule, Erfurt, Germany

(Received March 16/May 27, 1993)

Summary: Cortisol was determined in the sera of 687 normal, healthy probands between the ages of 5 days and 18 years (neonates, infants, children and adolescents), using a fluorescence polarization immunoassay (FPIA) on the TDx from Abbott Laboratories. The proband collective was divided into 9 age groups and each group into males and females. In accordance with the recommendations of the International Federation of Clinical Chemistry, the 95% scatter range was taken as the reference range. Only a few reference groups showed a normal *Gaussian* distribution. In addition to the 50th percentile, the 2.5th and 97.5th percentiles were determined for all reference groups. Minimal and maximal values were also determined. The U-test of *Mann & Whitney* was used to test for significant differences between the individual reference groups. Groups showing no significant differences were combined, and reference ranges were finally calculated for cortisol in the serum of normal, healthy neonates, infants, children and adolescents. The serum cortisol concentration showed a significant increase with age. In the 14–15 years age group, there was also a significant difference between males and females.

Introduction

Determination of plasma cortisol is indicated, if function tests of the hypothalamo-hypophyseo-adrenal cortex system suggest a deficient or excessive production of cortisol.

The serum cortisol concentration displays a circadian rhythm, with an increase in the early hours of morning and a marked decrease towards midnight (1–4). Determination of standard reference ranges is therefore difficult.

Since blood is normally taken for routine clinical purposes between 08.00 and 10.00 am, this time was chosen for determination of the reference ranges. The concentration measured at one particular time in the morning represents a single reference point, and must therefore be interpreted with caution. Further investigations are required to determine the daily profile of cortisol concentrations, in conjunction with different function tests.

The aim of the investigation was:

1) to determine the reference ranges for cortisol in normal, healthy neonates, infants, children and adolescents;
2) to test for significant sex differences in serum cortisol concentration within the age groups; and
3) to test for significant differences in serum cortisol concentration between the age groups.

Materials and Methods

Cortisol was determined in the sera of 687 subjects between the ages of 5 days and 18 years (neonates, infants, children and adolescents). Venous blood was taken from 5-day-old neonates during routine hypothyreosis screening. Blood was taken from all other probands with the written, informed consent of the parents; this procedure was agreed with the Ethics Commission of the Medical School, Erfurt.

The age composition of the proband collective is summarized in table 1. Individuals were included or excluded according to

Tab. 1. Age composition of the proband collective used for the determination of normal ranges of cortisol in neonates, infants, children and adolescents.

Group	Age	n
1 ♂	5th day	67
1 ♀	5th day	66
1	5th day	133
2 ♂	2–12 months	15
2 ♀	2–12 months	11
2	2–12 months	26
3 ♂	2– 3 years	18
3 ♀	2– 3 years	17
3	2– 3 years	35
4 ♂	4– 6 years	42
4 ♀	4– 6 years	24
4	4– 6 years	66
5 ♂	7– 9 years	44
5 ♀	7– 9 years	41
5	7– 9 years	85
6 ♂	10–11 years	44
6 ♀	10–11 years	53
6	10–11 years	97
7 ♂	12–13 years	45
7 ♀	12–13 years	47
7	12–13 years	92
8 ♂	14–15 years	39
8 ♀	14–15 years	38
8	14–15 years	77
9 ♂	16–18 years	38
9 ♀	16–18 years	38
9	16–18 years	76

the exclusion criteria of *Witt* and *Trendelenburg* (5), which permit the assembly of a reliable reference sample at justifiable expense. Only neonates with a birthweight between 2500 and 4000 g and a full term gestation time between 37 and 40 weeks were admitted to the 5-day-old age group. Neonates with hyperbilirubinaemia were excluded, as well as those born to mothers with acute or chronic illnesses.

Test material

About 2 ml of blood were taken from an arm or skull vein, between 08.00 and 10.00 am, using safety monovettes from Sarstedt, Nümbrecht. Blood samples were centrifuged immediately for 5 min at 3000 min^{-1}. The serum was removed with a pipette, then frozen at −22 °C until analysed.

Methods

Cortisol was determined by a fluorescence polarization immunoassay, with the TDx from Abbott Laboratories. The sensitivity of the test is reported to be 4.50 µg/l.

Quality control

For the control of precision from day to day, standards (from Abbott) of low, intermediate and high concentration were included intermittently in each series. As a measure of the relative methodical error, the arithmetic mean ($\bar{x}$), standard deviation (s) and the coefficient of variation (CV) were calculated from the individual results of the control series. Precision in series was monitored once, using the control sera, Serodos and Serodos Plus, from Greiner. Again, the arithmetic mean ($\bar{x}$), standard deviation (s) and coefficient of variation (CV) were calculated.

Statistical evaluation of the results

The results were first presented as separate histograms for each age group and for each sex. The type of distribution was determined with the *Kolmogorov-Smirnov* test. If the resulting probability error was below the stated value of $\alpha = 0.05$, the distribution was assumed to be normal. If the distribution was not normal, the 2.5th, 50th and 97.5th percentiles were determined for that reference group. In each age group, values were then tested for significant differences between the sexes, using the U-test of *Mann & Whitney*, with $\alpha = 0.05$ for the probability error. In the absence of a significant sex-related difference, males and females were subsequently treated as a single group. Age groups were again tested for significant differences with the U-test of *Mann & Whitney*, and those showing no significant difference were combined. The median value and reference range for the serum cortisol concentration were recalculated for all the new group combinations.

Results

Cortisol was determined in the serum of 687 probands (352 males, 335 females). Figure 1 gives an overview of the results for all groups before significance testing. A significant difference between the sexes was found in age group 8 ($p = 0.0169$). All reference groups were tested for significant differences, using the U-test of *Mann & Whitney*.

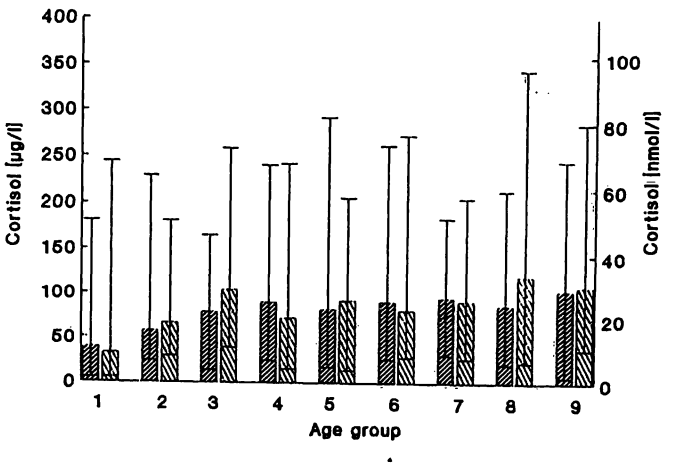

Fig. 1. Medians (50th percentiles) and 95% intervals for the concentrations of cortisol in serum (µg/l or nmol/l) in age groups 1–9 (see tab. 1).
▨ = males; ▩ = females.

Tab. 2. Median (50th percentile), 95% interval, minimal value and maximal value for the serum concentration of cortisol in neonates, infants, children and adolescents (values in µg/l).

Age	Sex	n	Median (50th percentile)	Reference (95% scatter) range (2.5–97.5th percentile)	Minimum	Maximum
5th day	♂/♀	133	33.3	5.5–198	4.50	313
2–12 months	♂/♀	26	64.4	23.8–229	23.8	229
2–13 years 14–15 years	♂/♀ ♂	413	87.9	25.1–229	13.4	299
14–15 years 16–18 years	♀ ♂/♀	114	108	24.2–286	6.30	343

Tab. 3. Results for the control of precision from day to day and in series.

	Control serum	n	$\bar{x}$ [µg/l]	s [µg/l]	CV [%]
Control from day to day	Abbott L	23	39.5	4.10	10.4
	Abbott M	30	150	11.6	7.75
	Abbott H	31	385	28.2	7.73
Control in series	Serodos	20	94.7	6.49	6.85
	Serodos Plus	20	553	25.7	5.64

The new group combinations, formed after significance testing, are shown in table 2. Median value and reference range for serum cortisol were recalculated for each new group combination.

Quality control

Results of the quality control are shown in table 3. The coefficients of variation within series and between series were all less than 10%.

Tab. 4. Reference ranges reported in the literature for cortisol in serum.

Author	Methods	Age groups	No. of probands	Type of distribution and scatter range	Reference range
Lashansky et al.*, 1991 (7)	RIA	<1 year	13	No data	342 ± 146
		1–5 years	14		337 ± 160
	[nmol/l]	6–12 years	8		254 ± 86
		Tanner 2–3	10	Median value	223 ± 74
		Tanner 4–5	13	given ± 1 SD	262 ± 80
		<1 year	8		353 ± 196
		1–5 years	8		281 ± 113
		6–12 years	7		229 ± 94
		Tanner 2–3	11		243 ± 102
		Tanner 4–5	8		279 ± 77
Rockicki et al., 1990 (12)	RIA	2 days	16	No data	2.93 ± 1.9
		3–5 days	19		2.29 ± 0.8
	[µg/dl]	6–12 days	10		4.87 ± 1.9
		13–20 days	6	Median value	4.38 ± 2.5
		2 months	3	given ± 1 SD	5.56 ± 2.0
		2 months	3		9.63 ± 3.1
Struckmeyer & Haid, 1986 (8)	RIA	<3 months	No clear data	No normal	11.1 (3.7 ± 16.4)
		3–6 months		distribution	13.8 (4.4–22.0)
	[µg/dl]	6 months–1 year			12.3 (4.9–19.9)
		1–6 years		Median value given	15.3 (9.1–21.6)
		6–16 years		with 90% scatter range (5%–95% quantile)	12.2 (4.7–19.6)
		<3months			11.0 (4.0–16.8)
		3–6 months			12.8 (4.9–19.2)
		6 months–1 year			11.3 (5.4–23.6)
		1–6 years			14.1 (7.3–20.9)
		6–16 years			12.6 (8.9–21.0)
*Forest**, 1978 (9)	RIA	1–4 months	10	No data	9.1 ± 4.8
		3–12 months	7		15.0 ± 5.6
	[µg/dl]	1–7 years	21	Median value	19.6 ± 7.4
		7–12 years	14	given ± 1 SD	19.2 ± 7.2

* The data represent the basal values reported in studies on the ACTH test in children

Discussion

The literature contains no reports of the determination of reference ranges for serum cortisol in children, using the present modern method (FPIA). Those childhood reference ranges that have appeared in the literature are shown in table 4 (6–9). These data are not comparable with the present results for the following reasons:

1) different methods were used;
2) the other authors used different age classifications;
3) the number of probands in the age groups of other authors were not given or they were very small;
4) exact data on the type of distribution of the reference values were not always given.

The 687 probands used in the present study represent an age range from the 5th day of life to the end of the 18th year. They were first divided into 18 groups according to age and sex (see tab. 1), based on the suggestions of *Egger* et al. (10) and the recommendations of the International Federation of Clinical Chemistry (11). Since neonates are subject to other diagnostic procedures (hypothyreosis screening) at the age of 5 days, this age was also chosen for the present investigation. No probands were available between the ages of 6 and 30 days. On account of the small sample size, the results for male and female infants in the age range 2–12 months have only limited value.

Since most reference groups did not show a normal distribution, the 2.5th and 97.5th percentiles were also calculated, in addition to the median value (12).

Cortisol concentrations were determined with a fluorescence polarization immunoassay (FPIA). This method has the advantage that the results are rapidly available, only a small sample volume is required, and radioactive isotopes are not used.

Cortisol concentrations in the serum of 5-day-old neonates were significantly lower than those of all other age groups. Any explanation for this low value must take into account the fact that cortisol displays no circadian rhythm at this age. These low serum cortisol concentrations in neonates are in agreement with the results of *Rockicki* et al. (6) and *Forest* (9). The work of *Struckmeyer & Haid* (8) showed no clear trend with age, whereas *Lashansky* et al. (7) found distinctly higher concentrations in infants.

In the present study, a significant difference between the sexes was found in the age group 14–15 years, but this was not found by any of the other authors.

Table 5 shows the reference ranges for serum cortisol as determined in the present study.

Tab. 5. Reference ranges for cortisol in the serum of neonates, infants, children and adolescents.

	µg/l	nmol/l
Males		
5th day	5.50–198	1.52–54.6
2–12 months	23.8 –229	6.57–63.2
2–15 years	25.1 –229	6.93–63.2
16–18 years	24.2 –286	6.68–78.9
Females		
5th day	5.50–198	1.52–54.6
2–12 months	23.8 –229	6.57–63.2
2–13 years	25.1 –229	6.93–63.2
14–18 years	24.2 –286	6.68–78.9

Acknowledgement

The authors wish to thank Mrs. *R. Biesenbach* for technical assistance. We also thank Abbott Laboratories for their generous support, which made this work possible.

References

1. New, M. I., Del Balzo, P., Crawford, C. & Speiser, P. W. (1990) The adrenal cortex. In: *Clinical Pediatric Endocrinology* (Kaplan, S. A., ed.) Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, W. B. Saunders Company.
2. Wallace, W. H. B., Crowne, E. C., Shalet, S. M., Moore, C., Gibson, S., Littley, M. & White, A. (1991) Episodic ACTH and cortisol secretion in normal children. Clin. Endocrinol. *34*, 215–221.
3. Krishnan, K. R. R., Ritchie, J. C., Saunders, W., Wilson, W., Nemeroff, C. B. & Carroll, B. J. (1990) Nocturnal and early morning secretion of ACTH and cortisol in humans. Biol. Psychiatry *28*, 47–57.
4. Linder, B. L., Esteban, N. V., Yergey, A. L., Winterer, J. C., Loriaux, D. L. & Cassoria, F. (1990) Cortisol production rate in childhood and adolescence. J. Pediatr. *117*, 892–896.
5. Witt, J. & Trendelenburg, C. (1982) Gemeinsame Studie zur Erstellung von Richtwerten klinisch-chemischer Kenngrößen im Kindesalter. J. Clin. Chem. Clin. Biochem. *20*, 235–242.
6. Rokicki, W., Forest, M. G., Loras, B., Bonnet, H. & Bertrand, J. (1990) Free cortisol of human plasma in the first three months of life. Biol. Neonate *57*, 21–29.

7. Lashansky, G., Saenger, P., Fishman, K., Gautier, T., Mayes, D., Berg, G., Di Martino-Nardi, J. & Reiter, E. (1991) Normative data for adrenal steroidogenesis in a healthy pediatric population: Age- and sex-related changes after adrenocorticotropin stimulation. J. Clin. Endocrinol. Metab. *73*, 674–686.
8. Struckmeyer, H. & Haid, H. (1986) *Richtwerte für das kinderärztliche Laboratorium*, 1. Auflage, Marburg. Die Medizinische Verlagsgesellschaft.
9. Forest, M. G. (1978) Age-related response of plasma testosterone, androstenedione, and cortisol to adrenocorticotropin in infants, children, and adults. J. Clin. Endocrinol. Metab. *47*, 931–937.
10. Egger, E., Dummler, W., Neymeyer, H.-G., Schwarze, H. & Scholz, R. (1987) Vorschlag zum Arzneibuch der DDR, Diagnostische Laboratoriumsmethoden, AB (D. L.)-DDR 89. Allgemeines, Referenzbereiche. Zbl. Pharm. *126*, 737–747.
11. International Federation of Clinical Chemistry Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values (1987) Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. J. Clin. Chem. Clin. Biochem. *25*, 639–644.
12. International Federation of Clinical Chemistry Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values and International Committee for Standardisation in Haematoloty. Standing Committee on Reference Values (1987) Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J. Clin. Chem. Clin. Biochem. *25*, 645–656.

Dr. med. Lars Jonetz-Mentzel
Doz. Dr. med. habil. Gudrun Wiedemann
Klinik und Poliklinik für Kindermedizin
der Medizinischen Hochschule Erfurt
Postfach 595
D-99012 Erfurt
Germany